Phytochemistry, Vol. 29, No. 8, pp. 2521–2524, 1990.
Printed in Great Britain.

0031-9422/90 $3.00+0.00

THE METABOLISM OF TROPINONE IN INTACT *DATURA INNOXIA* PLANTS*

MARY E. LANDGREBE and EDWARD LEETE†

Natural Products Laboratory, Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, U.S.A.

(*Received* 2 *January* 1990)

Key Word Index—*Datura innoxia*; Solanaceae; tropinone; hyoscyamine; scopolamine; tropine; alkaloids; biosynthesis; metabolism.

Abstract—[*methyl*-^{14}C]Tropinone was synthesized by reaction of nortropinone with [^{14}C]methyl iodide. This labelled ketone was administered to intact *Datura innoxia* plants (3–4-month-old) by addition to the nutrient solution in which the roots were growing. The resultant alkaloids isolated from the plants after two days were radioactive with high specific incorporations: hyoscyamine (2.5%), scopolamine (7.3%). Degradations of these alkaloids indicated that essentially all of the activity was present in their basic moieties and located on their *N*-methyl groups. These results are, thus, consistent with the hypothesis that tropinone is an intermediate in the biosynthesis of tropine from ornithine. The higher level of activity in the scopolamine indicates that the hyoscyamine is rapidly oxidized to this alkaloid.

INTRODUCTION

It is generally agreed that the biosynthesis of the tropane moiety of hyoscyamine (**7**) and scopolamine (**8**) is derived from hygrine (**1**) via the 5-acetonyl-1-methyl-Δ^1-pyrrolinium salt (**2**), tropinone (**3**) and tropine (**5**) [1] as illustrated in Scheme 1. The intermediacy of hygrine in this biosynthetic pathway is well established [2–6]. The iminium salt **2** is so far a hypothetical intermediate; however, biomimetic experiments [7] are consistent with this compound being the immediate precursor of tropinone. Tropinone has been found in several species: *Atropa belladonna* [8], *Crosstylis biflora* [9], *Cyphomatra betacea* [10] and *Nicandra physaloides* [11]. The reduction of tropinone has been studied in a cell-free system obtained from a root culture of *Datura stramonium* [12]. The crude enzyme, in the presence of NADPH, catalysed the formation of tropine. However, it was discovered [13] that a cell-free system, obtained from a root culture of *Hyoscyamus niger*, catalysed the reduction of tropine to ψ-tropine (**6**), NADPH being a cofactor. This was a surprising result as the major alkaloids of this species, hyoscyamine and scopolamine, are esters of tropine or its 6,7-epoxy derivative. Later [14] it was reported that a small amount of tropine (10–30%) was also formed in this enzyme system. It has been established that tropine is a precursor of hyoscyamine and scopolamine in *Datura meteloides* [15]. In these experiments, in which [*N*-methyl-^{14}C,3β-^{3}H]tropine was fed for seven days, there was no evidence of a reversible equilibrium between tropine and tropinone, because such a reaction would involve loss of tritium from the C-3 position of tropine.

The present article describes our preliminary experiments in which the metabolism of labelled tropinone in intact *Datura innoxia* has been investigated. Our work with a cell-free system obtained from the roots of this species will be described later.

RESULTS AND DISCUSSION

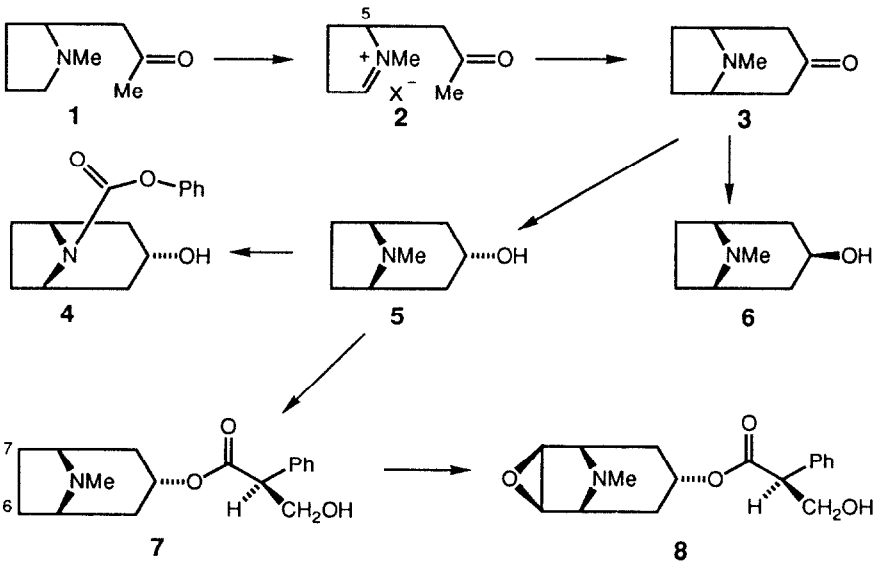
[*methyl*-^{14}C]Tropinone (**11**) was obtained by reaction of nortropinone (**10**) with [^{14}C]methyl iodide. Nortropinone was first prepared by Willstätter [16]; however, a more convenient synthesis was from commercially available tropinone, as illustrated in Scheme 2. Reaction of tropinone with phenyl chloroformate afforded *N*-phenoxycarbonylnortropinone (**9**). This compound was hydrolysed with potassium hydroxide to yield nortropinone.

The labelled tropinone was fed by addition to the hydroponic nutrient solution in which the roots of intact *Datura innoxia* plants (3–4-month-old) were growing. The absorption of the tropinone was rapid and the plants were harvested after two days. The alkaloids were extracted from the fresh plants as previously described [15]. Excellent incorporation of radioactivity into hyoscyamine and scopolamine (see Table 1) was obtained. The significantly higher specific activity of the scopolamine is consistent with the rapid metabolism of hyoscyamine to this epoxide [17, 18].

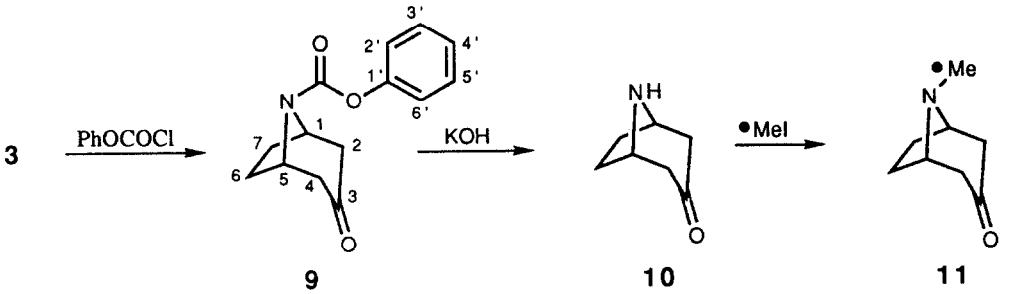
The location of radioactivity in the labelled alkaloids was established by chemical degradations. Diethyl azodicarboxylate has been used to demethylate tertiary *N*-methyl amines, the *N*-methyl group being oxidized to

*Contribution No. 217 from this laboratory. Part 46 in the series 'Chemistry of the Tropane Alkaloids and Related Compounds'.

†Author to whom correspondence should be addressed.



Scheme 1. Proposed biosynthesis of hyoscyamine and scopolamine from hygrine.



Scheme 2. Synthesis of [*methyl*-^{14}C]tropinone.

Table 1. Activities of hyoscyamine, scopolamine and their degradation products

Alkaloid	Activity (dpm mmol^{-1})
[*methyl*-^{14}C]Tropinone (**11**)	4.01×10^8
Hyoscyamine · HCl (**7**)	1.00×10^7
Specific inc. 2.5%	
Absolute inc. 2.4%	
Formaldehyde-dimedone*	0.86×10^7
Scopolamine · HCl (**8**)	2.94×10^7
Specific inc. 7.3%	
Absolute inc. 7.8%	
Hyoscyamine · HCl†	2.89×10^7
Tropic acid	$< 0.02 \times 10^7$
Tropine picrate	2.80×10^7
N-Phenoxycarbonylnortropine (**4**)	$< 0.05 \times 10^7$

*Derived from hyoscyamine.
†Derived from scopolamine.

formaldehyde [19]. The liberated formaldehyde was collected and assayed by preparation of its dimedone derivative. The specific activity of this derivative was 86% that of the hyoscyamine. This discrepancy from 100% may be due to the fact that the hyoscyamine was not completely radiochemically pure, or perhaps due to traces of formaldehyde being derived from other parts of the alkaloid, such as the hydroxymethyl group of the tropic acid moiety. The reaction of scopolamine with diethyl azodicarboxylate did not yield a significant amount of formaldehyde. The scopolamine was, thus, degraded by first converting it to hyoscyamine [20]. This was then hydrolyzed to tropic acid (negligible activity) and tropine. The latter was then demethylated by reaction with phenyl chloroformate affording *N*-phenoxycarbonylnortropine (**4**) [15] which had negligible activity.

Experiments were also carried out in which [*methyl*-^{14}C]tropinone was administered to intact *Datura innoxia* by means of cotton wicks inserted into the stems of the plants growing in soil. The incorporations were much poorer by this method of feeding (0.6% specific incor-

poration into hyoscyamine). However, a degradation on the hyoscyamine indicated that it was labelled specifically on its *N*-methyl group.

EXPERIMENTAL

General. Mp: corr. Radioactive materials were assayed by liquid scintillation counting using dioxane–EtOH as solvent with the usual scintillators [21]. All samples were counted in duplicate and the values reported in Table 1 are ±5%. GC was carried out on a 25 m glass capillary column coated with cross-linked Me silicone (0.52 μm thick) int diam 0.31 mm, using the following instrument parameters: He 1 ml min^{-1}, inj. temp. 250°, initial oven temp. 50°, equilibration time 4 min, rate of temp. increase 30° min^{-1}, final temp. 250°. GC retention times, (min), are recorded as GC R_t. NMR spectra were determined at 300 and 75.5 MHz respectively for ^1H and ^{13}C, with the assistance of Dr S. B. Philson. All recorded spectra are ppm from TMS. MS were determined by Dr E. Larka and his assistants at the University of Minnesota.

N-Phenoxycarbonylnortropinone (**9**). Phenyl chloroformate (3.0 ml, 24 mmol) was added to a solution of tropinone (3.0 g, 22 mmol) in dry CH_2Cl_2 (75 ml) and the mixture stirred at 25°C for 2 days. The residue obtained on evapn of the solvent was treated with 2 M HCl (30 ml) and the mixture extracted with Et_2O (30 ml × 20). The combined extracts were washed with 10% K_2CO_3. The residue obtained on evapn of this dried (Na_2SO_4) extract was crystallized from a mixture of EtOAc and petrol (bp 60–70°) to afford needles of *N*-phenoxycarbonylnortropinone (3.44 g, 65%) mp 123–124°, GC R_t 15.87. ^1H NMR ($CDCl_3$) δ1.74–2.81 (*m*, 8H, 2, 4, 6, 7), 4.64–4.72 (*br*, 2H, 1, 5), 7.12–7.38 (*m*, 5H, aromatic H); ^{13}C NMR ($CDCl_3$). Some of the chemically equivalent carbons appear as doublets due to restricted rotation of the amide bond, δ28.6, 29.5 (6, 7), 48.6, 49.4 (2, 4), 53.5 (1, 5), 121.6 (2′, 6′), 125.5 (4′), 129.4 (3′, 5′), 151.0 (1′), 151.8 (amide C=O), 207.4 (3). IR (KBr pellet) 3005, 2970, 2920, 2880, 1715 (ketone), 1595 (amide), 1495, 1400, 1210, 1195 cm^{-1}. EIMS (30 eV) *m/z* (rel. int.) 245 ($[M]^+$, 18), 152 (100), 110 (58), 109 (57), 94 (45), 81 (35), 77 (11), 67 (100), 41 (37). Anal. Calc for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.70; H, 6.25; N, 5.67%.

Nortropinone (**10**). *N*-Phenoxycarbonylnortropinone (2.2 g, 9 mmol) was dissolved in a mixture of 50% aq. KOH (30 ml) and EtOH (120 ml) and refluxed in a N_2 atmosphere for 24 hr. The cooled reaction mixture was dild with H_2O (90 ml) and extracted with $CHCl_3$ (100 ml × 6). The $CHCl_3$ extract was washed with 2 M HCl (100 ml × 4), evapd, and the residue dissolved in 10% aq. KOH (50 ml). The soln was extracted with $CHCl_3$ (75 ml × 6). The oil obtained on evapn of the dried (Na_2SO_4) extract was subjected to chromatography on a silica gel column. Nortropinone (393 mg, 35%) was obtained as a pale brown oil, on elution with a mixture of $CHCl_3$ and EtOH (9:1), GC R_t 9.67. Willstätter [16] reported this compound as needles, mp 69–70°. ^1H NMR ($CDCl_3$) δ1.14–2.50 (*m*, 8H, 2, 4, 6, 7), 3.79 (*br*, 2H, 1, 5). ^{13}C NMR ($CDCl_3$) δ30.1 (6, 7), 51.0 (2, 4), 54.9 (1, 5), 209.9 (3). IR (neat) 3400, 2970, 2890, 1705 (C=O) cm^{-1}. EIMS (30 eV) *m/z* (rel. int.): 125 ($[M]^+$, 15), 83 (18), 82 (46), 69 (15), 68 (100), 67 (80). High resolution MS: 125.0840, $C_7H_{11}NO$ requires 125.0841.

[methyl-^{14}C]*Tropinone* (**11**). [^{14}C]Methyl iodide (0.52 g, 3.7 mmol, nominal activity 1.5×10^9 dpm) in toluene (1.0 ml) was added to a solution of nortropinone (0.91 g, 7.28 mmol) in EtOAc (25 ml) and the mixture stirred at 25° for one day. The residue obtained on evapn of the yellowish-brown reaction mixture was dissolved in 10% NaOH (30 ml) and extracted with $CHCl_3$ (60 ml × 6). The residue obtained on evapn of the dried (Na_2SO_4) extract was subjected to chromatography on a column, (27 × 3 cm) of silica gel. Tropinone was eluted with CH_2Cl_2–MeOH–conc NH_4OH (85:15:1). Analytical TLC on silica gel GF-254, developing with the same solvent mixture, indicated that >99% of the radioactivity was coincident with a spot of authentic tropinone (R_f 0.6). The labelled tropinone (~300 mg), had a specific activity of 4.01×10^8 dpm $mmol^{-1}$. Further purification was achieved by HPLC on a prep. 10 × 250 mm column of silica gel 10 μ particle size, using the following gradient elution system: 100% $CHCl_3$ to 70% $CHCl_3$, 30% MeOH containing 1% conc. NH_4OH in 20 min, then to 100% MeOH with 1% conc. NH_4OH in 20 min, then to 100% MeOH with 1% NH_4OH for a total time of 1 hr. In a typical run tropinone eluted at 18 min. The [*methyl*-^{14}C]tropinone so obtained had a GC R_t of 9.88 min. The GC R_t of related compounds were: tropine 9.95, hyoscyamine 17.67, scopolamine 19.87 min. Decomposition of the last two alkaloids was avoided by the use of a lower (150°) inj. temp.

Administration of [methyl-^{14}C]*tropinone to* Datura innoxia *plants and isolation of the alkaloids.* [*methyl*-^{14}C]Tropinone (18.6 mg, 4.01×10^8 dpm $mmol^{-1}$, total act. fed: 5.97×10^7 dpm) was added to the nutrient solution (Murashige and Skoog basal salt mixture ($mg\,l^{-1}$): NH_4NO_3 (1650), H_3BO_4 (6.2), $CaCl_2$ (332), $CoCl_2 \cdot 6H_2O$ (0.025), $CuSO_4 \cdot 5H_2O$ (0.025), di-Na salt of EDTA (37.3), $FeSO_4 \cdot 7H_2O$ (27.8), $MgSO_4$(180.7), $MnSO_4$ (16.9), molybdic acid, di-Na salt·$2H_2O$ (0.25), KI (0.83), KNO_3 (1900), KH_2PO_4 (170), $ZnSO_4 \cdot 7H_2O$ (8.6), in which the roots of two intact *Datura innoxia* plants (3–4-month-old) were growing. Air was bubbled continously through this nutrient solution to keep the roots healthy. Uptake of radioactivity into the plants was rapid and after 24 hr only 2% remained unabsorbed. After 48 hr the plants (fr. wt 258 g) were harvested and extracted without drying as previously described [15]. The crude alkaloids (117 mg, 1.1×10^7 dpm, 18% of activity fed), when analysed by capillary GC, contained 37% of hyoscyamine and 44% of scopolamine. No peaks corresponding to tropinone, tropine and ψ-tropine were detected in the crude alkaloids. Prep. TLC afforded hyoscyamine and scopolamine which were purified by crystallization of their hydrochlorides. The activities of the alkaloids are recorded in Table 1.

Degradation of the hyoscyamine. Hyoscyamine·HCl (212 mg) was dissolved in H_2O (2 ml) which was made basic by the addition of NH_4OH. The mixture was extracted with $CHCl_3$ (3 × 5 ml). The residue obtained on evapn of the dried (Na_2SO_4) extract was dissolved in CH_2Cl_2 (7 ml) and diethyl azodicarboxylate (0.3 ml) added. After 18 hr the solution was evapd and the residue treated with 1 M HCl (20 ml). The mixture was then distilled, with replenishment of the H_2O into a solution of dimedone (350 mg) in water (100 ml). On standing for 18 hr the distillate deposited fine needles of formaldehyde-dimedone (38 mg, 20%), which was crystallized from MeOH.

Scopolamine was converted to hyoscyamine as previously described [20]. Hydrolysis of the hyoscyamine [22] afforded tropic acid and tropine. The tropine was demethylated to yield *N*-phenoxycarbonylnortropine mp 147–148° by reaction with phenyl chloroformate as described earlier [15].

Acknowledgement—This investigation was supported by research grants from the U.S. Public Health Service, National Institutes of Health (GM-13246) and the U.S. National Science Foundation (CHE-8620345).

REFERENCES

1. Leete, E. (1990) *Planta Med.* **55**, (in press).
2. Liebisch, H. W. and Schütte, H. R. (1967) *Z. Pflanzenphysiol.* **57**, 434.

3. O'Donovan, D. G. and Keogh, M. F. (1969) *J. Chem. Soc. (C)* 223.
4. McGaw, B. A. and Woolley, J. C. (1978) *Phytochemistry* **17**, 257.
5. McGaw, B. A. and Woolley, J. C. (1979) *Phytochemistry* **18**, 189.
6. McGaw, B. A. and Woolley, J. C. (1982) *Phytochemistry* **21**, 2653.
7. Leete, E. and Kim, S. H. (1989) *J. Chem. Soc. Chem. Commun.* 1899.
8. Hartman, T., Witte, L., Oprach, F. and Toppel, G. (1986) *Planta Med.* **52**, 390.
9. Medina, D. H. G., Pusset, G., Pusset, M. and Husson, H. P. (1983) *J. Nat. Prod.* **46**, 398.
10. Evans, W. C., Ghani, A. and Woolley, V. A. (1972) *J. Chem. Soc. Perkin Trans I* 2017.
11. Romeike, A. (1965) *Naturwissenschaften* **52**, 619.
12. Koelen, K. J. and Gross, G. G. (1983) *Planta Med.* **44**, 227.
13. Dräger, B., Hashimoto, T. and Yamada, Y. (1988) *Agric. Biol. Chem.* **52**, 2663.
14. Hashimoto, T. and Yamada, Y., unpublished work, cited by Yamada, Y., Hashimoto, T., Endo, T., Yukimune, Y., Kohno, J., Hamaguchi, N., Dräger, B. (1989) *Ann. Proc. Phytochem. Soc. Eur.* (in press).
15. Leete, E. (1972) *Phytochemistry* **11**, 1713.
16. Willstätter, R. (1896) *Ber.* **29**, 1575.
17. Hashimoto, T. and Yamada, Y. (1986) *Plant Physiol.* **81**, 619.
18. Hashimoto, T. and Yamada, Y. (1987) *Eur. J. Biochem.* **164**, 277.
19. Hosztafi, S. (1987) *Sci. Pharm. (Hung.)* **55**, 61.
20. Leete, E., Endo, T. and Yamada, Y. (1990) *Phytochemistry* **29** (in press).
21. Friedman, A. R. and Leete, E. (1963) *J. Am. Chem. Soc.* **85**, 2141.
22. Leete, E. (1987) *Can. J. Chem.* **65**, 226.